

## OBSERVATIONS ON THE POSSIBLE ROLE OF NUCLEIC ACID EXCHANGE REACTIONS IN PNEUMOCOCCAL CAPSULAR TYPE TRANSFORMATION

### A PRELIMINARY NOTE

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The majority of experiments concerned with the transformation of pneumococci from one capsular type to another have included several steps. In the first of these reactions, an unencapsulated mutant is derived by selective cultural techniques from a type-specific, encapsulated strain of pneumococcus. The selected mutant, which fails to produce capsular polysaccharide of any type, is transformed then in the presence of the desoxyribonucleic acids of an encapsulated pneumococcus of heterologous or homologous type and acquires thereby the ability again to produce a capsule. In an experiment of this kind, the type of capsular polysaccharide produced by the transformed cells is determined by the capsular type of the pneumococci from which the transforming principle is obtained.

The possibility that pneumococci of one capsular type may be transformed directly to a second type without the intermediation of the unencapsulated variant of the strain to be transformed is an interesting one though few data pertaining to this subject are available. In 1931, before many details of the transformation reaction were known, Dawson and Warbasse (1) attempted capsular type transformation of inocula of encapsulated pneumococci. Using an encapsulated strain of pneumococcus type II grown in a medium containing type I anticapsular rabbit serum and a transforming vaccine of heat-killed type III pneumococci, they succeeded in recovering occasional colonies of pneumococcus type III from platings of the original culture. Although the development of competent, unencapsulated variants of the type II strain during the course of the experiment could not be excluded, the authors considered it possible that direct transformation from capsular type II to type III had occurred.

Two later observations by Taylor have provided information which seems pertinent to the problem investigated initially by Dawson and Warbasse. In the first of these observations (2), it was noted that the diffusely growing,

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diplococcal, unencapsulated variant of pneumococcus (R) and the autoagglutinable, chain-forming, unencapsulated variant (ER) could be transformed reciprocally. To explain this phenomenon, Taylor suggested that an exchange reaction might take place between desoxyribonucleic acids within the cell and those contained in the transforming principle in its environment. If the hypothesis is correct and representative of a general biological phenomenon, then it would be reasonable to anticipate that capsular transformation could take place in an analogous fashion. In a second series of observations concerned with the hereditary control of the quantitative aspects of capsular polysaccharide production in pneumococcus type III (3), Taylor has shown it possible for encapsulated pneumococci to participate in transformation reactions under conditions which preclude largely, if not entirely, the intermediation of unencapsulated variants. This result also supports indirectly the possibility that encapsulated pneumococci might be transformed directly to an heterologous capsular type.

Recently experiments describing "the induction of heritable new type in type-specific strains of *H. influenzae*" have been reported by Alexander and Leidy (4). These experiments were performed in the presence of anticapsular serum directed against the strain of *H. influenzae* to be transformed, a procedure which, in pneumococcus, creates selection pressure favoring the development of unencapsulated forms.

The experiments to be reported here describe capsular type transformation in pneumococcus carried out with inocula of fully encapsulated strains and under conditions not favoring the development of unencapsulated variants.

#### MATERIALS AND METHODS

1. *Preparation of Transforming Extracts, Anti-R Serum and Transformation Reactions in Vitro.*—The techniques employed were those described by MacLeod and Krauss (5).

2. *Crystalline Desoxyribonuclease.*—Five mgm. of crystalline bovine pancreatic desoxyribonuclease (Worthington Biochemical Sales Co. lot D333) were dissolved in 10 cc. of charcoal-absorbed broth containing 0.02M  $\text{MgSO}_4$ . The enzyme solution was sterilized by passage through a Swinny filter and stored at 4°C. The stock solution was diluted prior to use.

3. *Strains of Pneumococcus.*—II-D39S: A fully encapsulated strain of pneumococcus type II.

R36NC, R36NDH5: two unencapsulated variants of the type II strain, II-D39S.

I-DRM, III-A66, III-A66RM, VIII-B: fully encapsulated strains of pneumococcus types I, III, and VIII, respectively.

R36NC-TVIII, R36NC-TVIII: fully encapsulated strains of pneumococcus type II and type VIII, respectively, obtained by transforming strain R36NC with the transforming principles of strain II-D39S or strain VIII-B.

R36NDH5-TVIII: a fully encapsulated strain of pneumococcus type VIII obtained by transforming strain R36NDH5 with the transforming principle of pneumococcus VIII-B.

## EXPERIMENTAL

To ensure insofar as possible the participation in the experiments of encapsulated pneumococci capable of being transformed, the two competent unencapsulated strains, R36NC and R36NDH5 were transformed to capsular types II or VIII. Inocula from single clones of the transformed encapsulated strains were seeded then into tubes containing 2 cc. of charcoal-absorbed broth, 5 per cent human pleural fluid agglutinating unencapsulated pneumococci, and transforming principle derived from an encapsulated pneumococcus of heterologous capsular type. In each experiment, control tubes lacking transforming principle were included. The cultures, which contained no anticapsular serum of any type at this time and therefore grew diffusely, were incubated for 24 hours at 37°C. After this initial incubation period, a loopful of each culture was streaked on a blood agar plate and quellung preparations were made. Examination of the plated subcultures revealed only the typically mucoid colonies of fully encapsulated pneumococci and the quellung preparations showed only

TABLE I  
*Biological Activity of Desoxyribonuclease on Pneumococcal Transforming Principles*

STRAIN	TRANSFORMING PRINCIPLE	DESOXYRIBONUCLEASE	CAPSULAR TYPE
R36NC	I-DRM	+	—
		—	I
R36NC	II-D39S	+	—
		—	II
R36NC	III-A66	+	—
		—	III
R36NC	III-A66RM	+	—
		—	III

encapsulated pneumococci of the type used to inoculate the culture. After these controls had been made, 10 $\gamma$  of crystalline desoxyribonuclease in 0.2 cc. of charcoal-absorbed broth were introduced into each tube and the tubes were reincubated for one hour at 37°C. Simultaneously a test of enzymatic activity was carried out to ensure the ability of the desoxyribonuclease to inhibit the transformation of the unencapsulated pneumococcus, R36NC, in the presence of the transforming principle employed in the experimental tubes. As recorded in Table I, the inhibition of transforming activity in each instance was complete. After the incubation of the experimental tubes in the presence of desoxyribonuclease, 1.6 cc. of each culture was transferred to a second tube containing 4 cc. of 10 per cent anticapsular rabbit serum directed against the inoculated strain in neopeptone broth. The cultures were incubated overnight and the diffuse growth in the supernatant fluid was examined by plating and quellung techniques. In each instance, as shown in Table II, cells of the same capsular type as the cells from which the transforming principle in the experimental

tube had been obtained were demonstrated. Study of the plated subcultures in one instance revealed the presence of rare colonies of unencapsulated pneumococci in the agglutinated precipitate, but these forms were noted only after destruction of the transforming principle by desoxyribonuclease and incubation of the contents of the experimental tube in the presence of homologous anticapsular serum.

TABLE II

*Pneumococcal Capsular Type Transformations Occurring After Inoculation of Encapsulated Strains*

INOCULUM	TRANSFORMING PRINCIPLE	CAPSULAR TYPES RECOVERED
R36NC-TII	III-A66RM	II, III
R36NC-TVIII	III-A66	VIII, III
R36NDH5-TVIII	I-DRM	VIII, I
	II-D39S	VIII, II
	III-A66	VIII, III

## DISCUSSION

The experiments reported here confirm the observation of Dawson and Warbasse (1) that encapsulated pneumococci may undergo capsular type transformation when grown in the presence of the transforming principle obtained from organisms of heterologous capsular type. Whether the reaction takes place as a result of the exchange of desoxyribonucleic acid within the cell for that introduced into the environment or through the intermediation of spontaneously arising, unencapsulated mutants during the growth of the cultures cannot be stated with certainty. The participation in reciprocal transformations of the diplococcal (R) and chain-forming (ER) unencapsulated variants of pneumococcus described by Taylor (2) has been considered explicable on the basis of nucleic acid exchange reactions and it has been shown also by Taylor (3) that encapsulated pneumococci may participate in transformation reactions. Both observations lend credence to the idea that transformation of capsular type in pneumococcus may take place through the medium of nucleic acid exchange reactions. On the other hand, no technique appears available at present whereby the possible appearance and subsequent transformation of unencapsulated mutant pneumococci during the course of the experiment can be excluded. Although the inclusion of antibody against unencapsulated pneumococci in the experimental system maintains selective pressure against unencapsulated forms (5), it cannot preclude their transient appearance and development. Until some more precise method, which of itself does not exert selective pressure, becomes available for the detection of minute numbers of unencapsulated mutants arising during the growth of encapsulated forms, the mechanism of capsular transformation under the conditions described must remain uncertain. The same arguments would seem to apply with equal force to the

question of capsular type transformation in *H. influenzae* as described by Alexander and Leidy (4).

#### SUMMARY

The phenomenon of pneumococcal capsular type transformation in experiments employing encapsulated pneumococci as the inoculum is described and discussed.

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